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**Original Article** 

# Nutrient and antinutrient retention in indigenous white cassava gari and provitamin A biofortified yellow cassava gari fermented over different time periods

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#### Abstract

This study evaluated the interaction between changes in fermentation period and nutrient/antinutrient composition of indigenous white cassava (IWC) gari and provitamin A biofortified yellow cassava (pVABYC) gari. For both the cassava varieties, as the fermentation time was increased from 2 to 5 days, the moisture and fat contents increased significantly whereas the ash and fibre contents reduced significantly. Phytate level decrease by 15.8% and 10% in the IWC gari and pVABYC gari respectively due to increase in fermentation period from 2 to 5 days. Tannin reduced by 3.4% and 5.1% while cyanogenic potential decreased by 10% and 27.8% in the IWC gari and pVABYC gari respectively due to increase in fermentation period from 2 to 5 days. As the fermentation time increased from 2 to 5 days, the total carotenoids,  $\beta$ -carotene, and provitamin A carotenoids reduced by 20.7%, 22.2%, and 23.4%, respectively, for pVABYC and by 22.6%, 21.4%, and 20.7%, respectively, for IWC. The percentage retention of the total carotenoids,  $\beta$ -carotene, and provitamin A carotenoids in the gari samples obtained from the two cassava varieties decreased significantly with an increase in the fermentation time. In conclusion, as the fermentation time increases, pVABYC is found to have a better nutritional retention capacity than IWC. Thus, pVABYC gari retains more nutrient in addition to providing 100% more  $\beta$ -carotene than IWC.

**Keywords**: Biofortified cassava, Vitamin A deficiency, Provitamin A carotenoids, Anti-nutritional factors, Fermentation, Food processing

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#### Introduction

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Carotenoids in foods are good dietary sources of provitamin A for animals (Rodriguez-Amaya et al., 2006; Oluba et al., 2018; Oluba and Oredokun, 2018).

In the body, provitamin A carotenoids are converted to vitamin A through oxidative cleavage (Stahl and Sies, 2005). In addition to being a potent antioxidant, vitamin A is an essential micronutrient that performs a vital role in certain aspects of the immune function and



in the maintenance of visual acuity (De Moura et al., 2015). Vitamin A deficiency (VAD) impairs human vision and damages the immune, respiratory, and reproductive systems. Consequently, individuals suffering from VAD are characterised by weak immune systems and are at a more considerable risk of dying from measles, diarrhoea or malaria (De Moura et al., 2015). In developing countries, children under five years and women are the major culprit of VAD, and over 3 million under five children in Africa are affected each year, with as much as 10% of these children becoming either partially or totally blind (Arlappa et al., 2011).

VAD is observed to be prevalent in tropical regions of the world where cassava is a staple crop and forms a major component of the daily food intake (Shrimpton, 1989). Biofortification has been targeted as a key strategy for improving the vitamin A intake of the poor especially in the tropical regions, through biofortification of commonly consumed local crops. The indigenous cassava tuber is limited in its content of  $\beta$ -carotene (a pro-vitamin A). Toxicity due to over consumption of  $\beta$ -carotene is rare because in humans the metabolism of  $\beta$ -carotene to vitamin A is a highly regulated process (Tanumihardjo et al., 2008).

For consumption purposes, cassava tuber is either boiled, steamed or processed into gari or flour (lafu) (Okechukwu and Okoye, 2010). Gari, a gelatinized granular flour, is a fermented food product obtained from cassava roots (Ojo and Akande, 2013). In Nigeria and neighbouring West African states, gari forms the bulk of the daily meal for the majority of the population including children and women (Maziya-Dixon et al., 2015). As a food staple, gari is consume most times in form of eba (Adepoju et al., 2010).

In Nigeria and other cassava-consuming nations, cassava is traditionally consumed after processing. Several traditional food-processing and preparation techniques are employed at the household level to enhance the taste, colour, texture, flavour, and palatability of the cassava-based foods (Cardoso et al., 2005). Processing techniques like peeling, soaking, grating, fermentation, cooking, boiling, and frying have been used to significantly reduce the antinutrient content of foods. Gari preparation requires peeling, washing, grating, fermentation, and frying (Bamidele et al., 2014). On the other hand, fufu preparation requires the extended fermentation of cassava roots in water, dewatering, and boiling of the fermented paste (Fayemi and Ojokoh, 2012). These various traditional processing methods of dish

preparation not only affect several desirable attributes of the resulting food products but also alter the nutritional composition of the original food sources (Davis et al., 2009). Previous studies on the carotenoid content of cassava tend to focus on unprocessed food sources. However, the overall effect of nutrients and provitamin A carotenoids in foods on human health depends largely on the types of processing that such food sources have undergone prior to their consumption by a particular population (Maziya-Dixon et al., 2015; Maziya-Dixon et al., 2008). Furthermore, the retention and bioaccessibility of nutrients and provitamin A carotenoids in processed cassava products should constitute the basis for determining both the quality and quantity of food carotenoids that should be consumed (Rodriguez-Amaya et al., 2006; Boileau et al., 1998). Thus, more research should be conducted on the effect of processing on nutrients and provitamin A carotenoids, especially in biofortified cassava genotypes. Accordingly, this study was designed to evaluate the effect of different fermentation times on nutrient retention in gari processed from the indigenous white cassava (IWC) and provitamin A-biofortified yellow cassava (pVABYC) varieties grown in Nigeria. The overall aim of this study was to improve the nutritional status of VAD-challenged populations by providing research-based guided information that could aid their choice of cassava genotypes with adequate nutrient and provitamin A carotenoids bioavailability.

#### **Material and Methods**

#### **Plant materials**

Tubers of the two cassava varieties-wild type cultivar, TMS 30572 (IWC) and TMS 07/593 (pVABYC) were obtained from the crop multiplication unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Authenticated of the cassava varieties was done by Mr. Kehinde Oyebanji, a taxonomist in the Department of Crop Science, Joseph Ayo Babalola University, Ikeji Arakeji, Osun State, Nigeria.

#### Gari processing

Freshly harvested roots (120 kg) each of both IWC (TMS 30572) and pVABYC (TMS 07/593) were manually peeled using a knife, thoroughly washed with tap water and grated. The grated mash for each cassava varieties was divided into two equal batches and packed separately in clean white polypropylene sack, covered with black nylon to reduce light

exposure. The first batch for each cassava was left to ferment for 2 days while the second batch was fermented for 5 days. The fermentation took place under the same conditions (temperatures between 28 and 33 °C; 60 - 70% humidity). The fermented mash was thereafter dewatered, pressed into a solid cake using a hydraulic press, and sifted manually using a stainless-steel sieve. The sieved wet granules were fried in a large, shallow stainless-steel pan with constant stirring using a piece of thick calabash at 160-170 °C for 10 min. The fried gari was allowed to cool, packed in an aluminium foil, and stored at room temperature until it was required for further analysis. The different operations involved in the processing of cassava roots to produce gari in this study are shown in Fig-1.



Figure-1: Unit operations involved in processing of gari from cassava tuber

#### Nutritional composition analyses Proximate composition

The percentage moisture, ash, fat, fibre, and crude protein contents of the 2- and 5-day fermented gari samples obtained from the two cassava cultivars were analysed following standard methods (AOAC, 2005). The percentage total carbohydrate content was obtained by difference. The sugar and starch contents were analysed using the methods of Juliano et al. (1981). All the determinations were carried out in triplicates.

#### Anti-nutritional factor

The antinutritional factors in raw cassava and gari samples were analysed according to AOAC (2005).

#### **Carotenoid analysis**

The whole root (grated raw sample) and gari samples were analysed for total carotenoid content according to the method of Rodriguez-Amaya and Kimura (2004) and described by Oluba et al. (2018).

#### Statistical analysis

Results are mean  $\pm$  SD or standard error of mean (SEM). Statistical comparisons between means were done using paired Student's t-tests or one-way ANOVA, as applicable while p < 0.05 was considered significant.

#### **Results**

#### **Proximate composition**

Moisture content of the gari samples processed from the two cassava varieties was significantly lower compared to the raw cassava (IWC and pVABYC). The raw samples of both IWC and pVABYC were significantly lower in ash contents compared to their respective gari products. The 2-day fermented pVABYC gari had the highest ash content  $(1.7 \pm 0.1)$ . The fat content of the raw cassava samples (IWC and pVABYC) was significantly lower compared to their respective gari products. The fat level in the 5-day fermented gari was significantly higher than the 2-day fermented gari for both cassava varieties (Table 1). The raw samples for both cassava varieties were significantly lower in crude fiber compared to their respective gari samples. The pVABYC gari samples were observed to show significantly higher crude fiber content compared to IWC gari samples. The unprocessed IWC and pVABYC showed significantly lower protein content compared to the processed gari products. The 5-day fermented pVABYC gari had the highest protein content (0.61  $\pm$  0.01/100 g DW). Though the raw IWC and pVABYC samples were significantly lower compared to their finished gari products no significant difference was observed in the total carbohydrate level due to increase in fermentation period from 2 to 5 days for both cassava varieties.

Proximate Composition (%)	IWC (raw)	IWC gari (2DF)	IWC gari (5DF)	pVABYC (raw)	pVABYC gari (2DF)	pVABYC gari (5DF)
Moisture	$66.8\pm9.98^{b}$	$7.17\pm0.01^{a}$	$9.43\pm0.01^a$	$54.77\pm7.82^{b}$	$7.43\pm0.01^{a}$	$8.88\pm0.02^{a}$
Ash	$0.46\pm0.03^{\rm a}$	$0.99\pm0.01^{\text{b}}$	$1.19\pm0.01^{c}$	$0.48\pm0.02^{a}$	$1.7\pm0.1^{d}$	$0.87\pm0.02^{b}$
Fat	$0.48\pm0.02^{\rm a}$	$0.92\pm0.02^{\rm c}$	$1.26\pm0.02^{d}$	$0.56\pm0.04^{b}$	$0.96\pm0.02^{\rm c}$	$1.28\pm0.04^{\rm d}$
Fibre	$0.82\pm0.03^{\rm a}$	$2.92\pm0.06^{d}$	$2.6\pm0.1^{\circ}$	$1.1\pm0.06^{b}$	$5.6\pm0.0^{\rm f}$	$4.46\pm0.0^{e}$
Protein	$0.25\pm0.04^{a}$	$0.44\pm0.05^{\circ}$	$0.44\pm0.06^{c}$	$0.33\pm0.02^{a,b}$	$0.42\pm0.02^{b,c}$	$0.61\pm0.01^{\rm d}$
Total carbohydrate	$35.16\pm5.30^a$	$86.84\pm0.25^{b}$	$83.44\pm0.35^b$	$43.06\pm0.10^{a}$	$83.39\pm0.09^{b}$	$81.38\pm0.10^{b}$
Sugars	$3.10\pm0.2^{\rm a}$	$4.46\pm0.13^{\text{c,d}}$	$3.79\pm0.08^{b}$	$7.85\pm0.13^{\rm e}$	$4.91\pm0.2^{\text{d}}$	$4.37\pm0.25^{\rm c}$
Starch	$32.55\pm0.05^{a}$	$77.15\pm0.05^{\rm f}$	$70.5\pm0.5^{\text{d}}$	$35.30\pm0.2^{b}$	$73.8\pm0.82^{e}$	$63.75\pm0.13^{\rm c}$
Amylose	$9.1\pm0.2^{\rm a}$	$24.86\pm0.0^{\text{d}}$	$26.12\pm0.11^{e}$	$10.1\pm0.44^{b}$	$21.78\pm0.0^{c}$	$25.34\pm0.06^{d}$
Amylopectin	$23.45\pm0.25^a$	$50.6\pm0.36^{e}$	$44.4\pm0.44^{d}$	$25.18\pm0.11^{b}$	$53.58 \pm 0.04^{\rm f}$	$38.4\pm0.35^{\rm c}$

 Table-1: Effect of fermentation time on proximate composition of raw IWC and pVABYC genotypes and their respective gari products

Values are Means  $\pm$  SD of three independent determinations. Mean with different superscript letters in column are significantly different (p < 0.05). Note: IWC, indigenous white cassava; pVABYC, provitamin A-biofortified yellow cassava; 2DF, fermented for 2 days; 5DF, fermented for 5 days.

The raw cassava sample for the IWC had the least sugar level  $(3.10 \pm 0.2)$  while the pVABYC (raw) had the highest sugar level  $(7.85 \pm 0.13)$ . The raw samples of the two cassava varieties was significantly lower in starch and amylopectin contents compared to their respective gari samples. The 2-day fermented gari samples for both cassava varieties were significantly higher in starch and amylopectin levels compared to the 5-day fermented gari. The amylose content in the raw samples for both cassava varieties was significantly lower compared to their respective gari samples. The 5-day fermented IWC gari had the highest amylose content ( $26.12 \pm 0.11$ ) (Table 1).

#### **Anti-nutritional factors**

Phytate level in gari processed from the IWC was significantly reduced when fermentation period was increased from 2 days to 5 days. Similarly, a significant decrease in phytate level was seen in the 5day fermented pVABYC gari compared to the 2-day fermented pVABYC gari. A comparative analysis in the phytate level between the 2-day fermented gari products from the two cassava varieties showed a significant increase in the pVABYC gari compared to the IWC. However, phytate levels in the 5-day gari products from the two cassava varieties was not significantly altered. Increase in fermentation period from 2 to 5 days was observed to significantly reduce levels of tannin in gari samples from the two cassava varieties. Tannin concentration between the 2-day, as well as 5-day fermented gari samples from the two

cassava varieties was not significantly altered. Fermentation period had no significant effect on the cyanogenic potential of gari samples processed from the IWC. However, increase in fermentation time from 2 to 5 days produced a significant decrease in cyanogenic potential level in the pVABYC gari cultivar. A comparative analysis of the cyanogenic potentials for gari products from the 2-day and 5-day fermentation processes in the two cassava varieties showed that the pVABYC gari contained significantly higher levels compared to the IWC (Fig. 2).



### Figure-2: Effect of fermentation time on antinutritional factors of gari obtained from the IWC and pVABYC

Values are Means  $\pm$  SD of three independent determinations. Bar carrying different alphabets are significantly different (p < 0.05). Note: IWC,

Asian J Agric & Biol. 2020;8(1):44-51

indigenous white cassava; pVABYC, provitamin Abiofortified yellow cassava; 2DF, fermented for 2 days; 5DF, fermented for 5 days.



### Figure-3: Effect of fermentation time on carotenoids content of gari obtained from the IWC and pVABYC

Values are Means  $\pm$  SD of three independent determinations. Note: IWC, indigenous white cassava; pVABYC, provitamin A-biofortified yellow cassava; 2DF, fermented for 2 days; 5DF, fermented for 5 days.



Figure-4: Carotenoid retention in gari product of the IWC and pVABYC fermented for 2 and 5 days Values are Means  $\pm$  SD of three independent determinations. \* Significantly different (p < 0.05). Note: IWC, indigenous white cassava; pVABYC, provitamin A-biofortified yellow cassava; 2DF, fermented for 2 days; 5DF, fermented for 5 days.

#### Carotenoids

The chromatograms for the carotenoid array of the IWC gari products fermented for 2 and 5 days are shown in Supplementary Figs. 1a and 1b, respectively. Similarly, those of the pVABYC gari products fermented for 2 and 5 days are shown in

Supplementary Figs. 2a and 2b, respectively. Significant reductions of 20.7%, 22.2%, and 23.4% for pVABYC and 22.6%, 21.4%, and 20.7% for IWC in the total carotenoids,  $\beta$ -carotene, and provitamin A carotenoids, respectively, were observed due to increase in the fermentation time from 2 to 5 days (Fig. 4). Furthermore, the retention of carotenoids in the gari samples because of the increase in the fermentation time from 2 to 5 days with respect to unprocessed cassava roots revealed significant decreases (p = 1.0) in both cassava varieties (Fig. 3).

#### Discussion

The study results showed that an increase in the fermentation time from 2 to 5 days resulted in a significant increase in the moisture content of gari samples obtained from both IWC and pVABYC thus increasing its hygroscopic property. The moisture content of cassava products is a function of several variables including temperature, time, humidity, and season (Achinewhu et al., 1989). If the moisture content is low, gari stay longer in the shelf because it is less prone to microbial spoilage, which would otherwise comprise its quality (Oluba et al., 2018; Irtwange and Achimba, 1990). An increase in the fermentation time from 2 to 5 days was also observed to enhance the crude fat and protein contents of gari obtained from the two cassava varieties. These findings agree closely with the results of Oboh et al. (2002), who observed that the crude fat level in cassava flour and gari increased by 5.7% and 4.0%, respectively, when fermented with Aspergillus niger. The crude protein content in the gari samples fermented for 5 days was significantly higher than that in the gari samples fermented for 2 days. However, the observed crude protein contents for both the 2- and 5day fermented gari in this study were low compared to the content values reported by Oboh et al. (2002), Oboh and Akindahunsi (2003), and Ruthairat et al. (2010). The increase in the crude protein content of gari could be attributable to the release of some bound protein due to breaking of bonds such as peptide bond and hydrogen bond due to longer fermentation time. The starch matrix of the fermenting cassava broth is degraded into utilizable carbon sources and simultaneously converted to proteinaceous materials to sustain the microbial growth and development (Ruthairat et al., 2010). In addition, the growth and multiplication of the bacterial and fungal cells in the

fermenting cassava broth in the form of single cell protein could contribute to the observed increase in the protein content of the gari samples. Furthermore, the observed increase in the protein content of fermented gari in this study could be attributed to the loss of dry matter, mainly carbohydrates (Ruthairat et al., 2010). Our data also revealed that the crude fibre content in the gari products obtained after fermentation for 5 days was greater than that in the gari products obtained after fermentation for 2 days. This observation concurs with the findings of Oboh et al. (2002), who reported that the crude fibre content in cassava flour and gari were 5.7% and 4.0%, respectively. Similarly, Oboh and Akindahunsi (2003) reported increased crude fibre contents of 4.5% and 3.0% for cassava flour and gari, respectively. The observed increase in the crude fibre content of gari in this study could be attributed to the conversion of the available carbohydrates to fat by microorganisms present in the fermentation broth (Krisada et al., 2009). Some microorganisms have also been reported to produce microbial oil during fermentation (Irtwange and Achimba, 1990; Eugene et al., 2008). The total carbohydrate content of gari fermented for 5 days was observed to be less than that of gari fermented for 2 days; however, the difference was not significant. This trend was similar to the observation of Akindahunsi et al. (1999), who reported that the carbohydrate level in cassava products fermented with Saccharomyces cerevisiae and Lactobacillus bulgaricus was non-significantly less than that in naturally fermented products. The lower carbohydrate content could be due to the ability of fungal or bacterial biomass to hydrolyse starch into glucose, which acts as a carbon source in the synthesis of fungal or bacterial biomass rich in protein (Krisada et al., 2009). Previous investigations by Oboh and Akindahunsi (2003) and Aro and Aletor (2006) in a related study observed a similar trend.

Our study results revealed that the contents of antinutritional factors in the form of phytate, tannin, and cyanogenic glycosides in gari reduced significantly because of an increase in the fermentation time from 2 to 5 days for the two cassava cultivars. This observation was similar to the results of Rao and Deosthale (1982). The presence of phytic acid in plantbased food products has been demonstrated to interfere with the bioavailability of some mineral elements and inhibit protein utilisation because of its ability to form complexes with body proteins (Ferguson et al., 1989). Tannins in foods have been reported to interfere with the hydrolytic actions of some digestive enzymes such as trypsin, trypsinogen, amylases, and lipases, thus reducing the digestibility of dietary proteins, carbohydrates, and lipids (Rao and Deosthale, 1982). This has been shown to lead to growth depression in rats and poultry. The decrease in the phytic acid content could be due to the activities of microbial phytase.

The results obtained in this study showed that the carotenoid content of gari reduced drastically because of an increase in the fermentation time. In addition, the retained amounts of total carotenoids, β-carotene, and provitamin A carotenoids with respect to the amounts contained in the unprocessed cassava root decreased significantly when the fermentation time was increased from 2 to 5 days. Processing techniques involving slicing, grating, mashing, and drying, which most often increase the surface area of food or food products, have been reported to expose the carotenoids in food to light and air, whereas processes such as baking, boiling, and frying subject the carotenoids to the destructive effect of high temperatures (Thakkar et al., 2009). It is thus not surprising that processed plantbased food products often contain significantly lower carotenoids than the raw or unprocessed form of the same food. Some amounts of carotenoids are lost during the peeling stage of cassava roots as well as through isomerisation and enzymatic or nonenzymatic oxidation of trans-carotenoids (Rodriguez-Amaya and Kimura, 2004). The high degree of unsaturation of carotenoids makes them highly susceptible to isomerisation and oxidation. Chavez et al. (2007) reported that when cassava mash was fermented for 7 days, approximately 66% of βcarotene in the produced gari was lost. The percentage loss in total carotenoids for both 2- and 5-day fermented gari observed in this study was less than that observed in other similar studies; this could be attributed to the low frying or toasting temperature of 170 °C in the present study. Chavez et al. (2007) previously reported that close to 50% loss in the  $\beta$ carotene content of gari could be prevented by reducing the roasting temperature from 195 to 165 °C.

#### Conclusion

Data generated from the study revealed that the 2-day fermented gari products of the two cassava varieties retained more nutrients in ash, fat, fibre, protein, total carbohydrate, starch, amylose and amylopectin compared to their respective 5-day fermented gari. In addition, the 2DF gari had a better carotenoid

retention capacity when compared to the 5DF gari. Thus, with respect to provitamin A retention, prolonged fermentation time in the processing of cassava roots is discouraged to minimise nutrient loss.

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51